

DEPARTMENT OF THE INTERIOR  
U.S. FISH AND WILDLIFE SERVICE  
REGION 6

**ENVIRONMENTAL CONTAMINANTS PROGRAM**  
**ON-REFUGE INVESTIGATIONS SUB-ACTIVITY**

**FINAL REPORT: YEAR 6**  
**Nationwide Malformed Amphibian Monitoring Project**

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## Executive Summary

In 2005, U.S. Fish and Wildlife Service (Service) personnel monitored five National Wildlife Refuges (NWRs) in Region 6, which included Alamosa NWR and Arapaho NWR in Colorado, Rainwater Basin NWR in Nebraska, Lostwood NWR in North Dakota, and Mortenson NWR in Wyoming, for the presence of abnormal amphibians. This was the second year of monitoring at each of these refuges.

Overall, there were few abnormalities found in amphibians. In 2004, Lostwood NWR was the refuge with the highest number of abnormalities in metamorphs, but in 2005 no metamorphs were found. At the Rainwater Basin NWR in Nebraska abnormal metamorphs were found at two sites in 2005 compared to five sites in 2004. Abnormalities were found in four Plains leopard frog (*Rana blairi*) metamorphs and in three metamorphs of an unknown toad species (*Bufo* sp.) Several sites at two refuges in Colorado were monitored for abnormal amphibians. Four abnormal chorus frog (*Pseudocris triseriata*) metamorphs were found at one site on Arapaho NWR. At Alamosa NWR, only one abnormal chorus frog metamorph was found. Reconnaissance was conducted in 2004 and 2005 at the nearby Monte Vista NWR to identify if suitable amphibian breeding habitat was available. Although chorus frog tadpoles and Woodhouse's toad (*Bufo woodhousii*) tadpoles were observed, no metamorphs were found. It is presumed that limited water and breeding habitat combined with heavy predatory pressure limit amphibian abundance at this refuge. At Mortenson NWR in Wyoming, four chorus frog metamorphs were found to have abnormalities in 2004 but only one metamorph was found in 2005.

The abnormal specimens found during the monitoring effort were sent to the University of Wisconsin for parasitological examinations as some parasites may be responsible for certain malformations. Upon completion of the parasitological examinations, the metamorphs were preserved and sent to Ball State University for radiography. Results of the examinations and radiographs are pending.

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### **Attached Appendices**

Appendix A1. Monitoring report for Alamosa and Arapaho NWRs, site map, photos, and data collection forms for Arapaho NWR, Colorado.

Appendix A2. Site map, photos, and data collection forms for Alamosa NWR, Colorado.

Appendix A3. Site map, photos, and data collection forms for Monte Vista NWR, Colorado.

Appendix B. Monitoring report and photos for Rainwater Basin NWR, Nebraska.

Appendix C. Monitoring report for Lostwood NWR, North Dakota.

Appendix D. Monitoring report, site map, photos, and data collection forms for Mortenson NWR, Wyoming.

## INTRODUCTION

In recent years, an increasing number of frogs have exhibited severe abnormalities. Because of the apparent increase in amphibian abnormalities and reports that such amphibians appear on Federal Lands, the Department of Interior distributed funding to the U.S. Fish and Wildlife Service (Service) to confirm the presence of abnormal frogs on National Wildlife Refuges (NWRs).

The purpose of the monitoring effort is to: determine if abnormal amphibians occur on Service lands; develop a database on land use surrounding selected Refuges; and contribute to the overall knowledge of regional and national distribution of the phenomenon. Monitoring focuses on examining 50 - 100 newly metamorphosed frogs, preferably *Rana* species, for abnormalities at two or more sites on a refuge. A newly metamorphosed species of frog is defined as having a snout-vent length (SVL) of less than 50 mm and completing Gosner stage 46 (Gosner 1960). The effort typically requires a minimum of three people for capturing etamorphs and collecting the necessary data. At sites where *Rana* species are not present in numbers necessary to complete the monitoring, data was collected on other species of frogs (*Hyla* sp.) and toads (*Bufo* sp.). Any abnormal amphibians collected during monitoring in 2000 - 2002 were photographed, preserved, and sent to the U.S. Geological Survey National Wildlife Health Center (NWHC) in Madison, Wisconsin, for further examination. In 2003, 2004, and 2005, abnormal amphibians collected during monitoring were sent alive to the University of Wisconsin at La Crosse for parasitology examinations. Upon completion of the parasitological exam, the metamorphs were preserved and sent to Ball State University for radiography.

Six refuges were selected for monitoring in the year 2000 and included: Lake Alice, North Dakota; Lake Andes and Sand Lake, South Dakota; Quivira, Kansas; Ouray, Utah; and Cokeville Meadows, Wyoming. Monitoring occurred at these same refuges in 2001 except for Lake Alice NWR where water levels were too high. Monitoring occurred instead at Long Lake NWR in North Dakota. Two additional refuges were monitored in 2001 (Valentine NWR in Nebraska and Lee Metcalf NWR in Montana). In 2002, those refuges that were monitored both in 2000 and 2001 and where the occurrence of abnormal frogs was <3% were not selected for a third year. However, Long Lake NWR, Lee Metcalf NWR, and Valentine NWR were monitored for a second year. In addition, Waubay NWR in South Dakota and Flint Hills NWR in Kansas were monitored in 2002. In 2003, monitoring occurred at the same refuges as in 2002. All new refuges were monitored in 2004. These refuges included Mortenson Lake in Wyoming, Lostwood in North Dakota, Rainwater Basin in Nebraska, and Arapaho and Alamosa in Colorado. These refuges were sampled again in 2005.

During monitoring at Long Lake Wetland Management District (District) at Long Lake NWR in 2002 and 2003, Service personnel followed the development of metamorphosing northern leopard frogs (*Rana pipiens*) by keeping them in captivity until metamorphosis was complete. The impetus for observing the development of these metamorphs came from the District's 2001 monitoring efforts. In 2001, seven northern leopard frog metamorphs with fully developed mouths and retained tails ranging from 16-42 mm in length were collected. According to the

Service's 2001 Frog Sampling Protocol "any tail >2 mm in length is considered retained if the frog's mouth is fully developed," and retained tails are considered abnormalities/malformations. These seven frogs were recorded as abnormal/malformed, euthanized, and preserved according to protocol. Subsequently, the District staff's curiosity was piqued regarding these "tailed metamorphs."

In 2002 and 2003, District staff investigated whether frogs having a mouth developed beyond their eye (Gosner stage #45) and having a retained tail >2 mm in length are abnormal/malformed, or simply a product of developmental variability (i.e., some frogs resorb their tail at a different rate than others). District staff decided all metamorphs with tails that were captured during the 2002 and 2003 monitoring efforts and that met the above criteria would be kept in captivity to monitor their continued development. This project allowed us to see if, given adequate time, frogs captured during the late stages of metamorphosis (i.e., the mouth is developed beyond the eye) and still having a tail >2 mm in length, would eventually resorb their tail. In 2002 and 2003, all frogs captured for this project did resorb their tails. Consequently, the presence of a retained tail was no longer considered an abnormality.

## SAMPLING LOCATIONS

The Mountain Prairie Region (Region 6) of the Service contains eight states (Figure 1). Detailed sampling location information of the five refuges monitored in 2005 is provided in Table 1. Site maps and photographs of sampling locations are included in the Appendices.

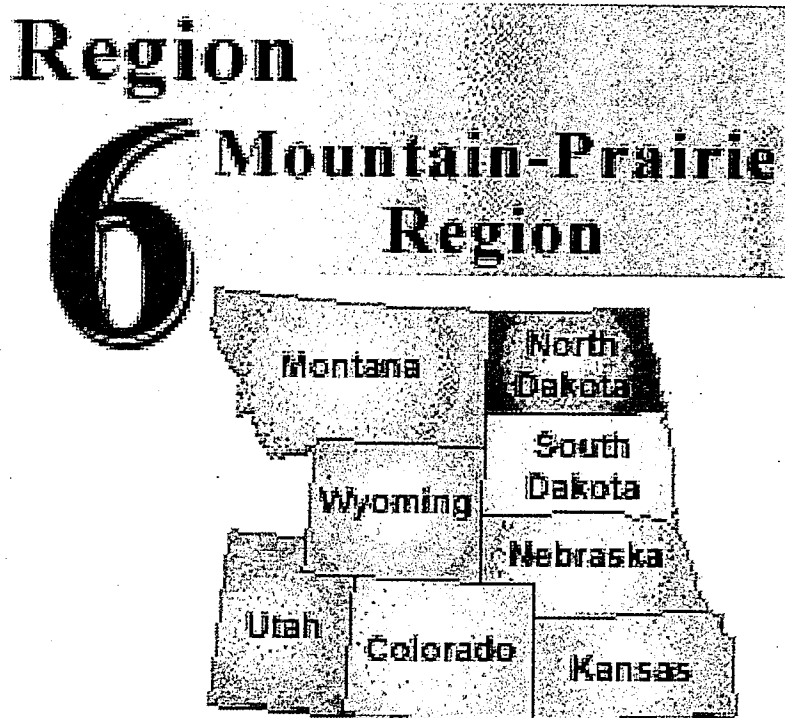


Figure 1. Mountain-Prairie Region of the U.S. Fish and Wildlife Service.

### *Colorado*

#### Arapaho National Wildlife Refuge

Arapaho National Wildlife Refuge lies within an intermountain glacial basin known locally as North Park. The refuge is located one mile south of the town of Walden, Colorado, in the northern part of the state. Arapaho NWR covers 24,804 acres and supports diverse wildlife habitats including sagebrush-grassland uplands, grassland meadows, willow riparian areas, wetlands, and mixed conifer and aspen woodlands. The elevation ranges from 8,100 to 8,700 feet. Average annual precipitation is 12 inches per year. Arapaho NWR was established in 1967 to provide suitable nesting and rearing habitat for migratory birds. Numerous wetlands have been developed or enhanced on the refuge, fed by irrigation water from the Illinois River. The

Illinois River, a tributary to the North Platte River, traverses from south to north through the eastern portion of the refuge. The refuge provides habitat for a variety of wildlife species including 198 species of birds, 32 species of mammals, 1 reptile and 5 amphibian species, and 9 species of fish. Land use surrounding the refuge includes cattle grazing, hay production, and lands administered by the BLM.

Amphibian species that occur in North Park are: striped chorus frog, northern leopard frog, wood frog (*Rana sylvatica*), western toad (*Bufo boreas*), and barred tiger salamander (*Ambystoma tigrinum mavortium*). Twelve new sites were monitored for metamorphs in 2005. The sites are primarily palustrine emergent wetlands. Vegetative cover is relatively lush at this site. The dominant cover species is Nebraska sedge (*Carex nebraskensis*); forbs include bluebell (*Mertensia ciliata*), lupine (*Lupinus argenteus*), spearmint (*Mentha spicata*), curly dock (*Rumex* sp.), false Solomon's seal (*Smilacina stellata*), and elephantella (*Pedicularis groenlandica*). Willow (*Salix* sp.) shrubs are scattered throughout the site, and become dense toward the east side of the site along the Illinois River. Submergent vegetation includes pondweed (*Potamogeton* sp.), mare's tail (*Hippuris vulgaris*), and water milfoil (*Myriophyllum* sp.). The surrounding land uses include riparian corridor, on- and off-refuge cattle grazing, and hay production.

#### Alamosa National Wildlife Refuge

Alamosa NWR is located in the San Luis Valley, approximately 4 miles east of the town of Alamosa, Colorado, in the southern part of the state. Alamosa NWR covers 11,169 acres and lies within the Rio Grande River floodplain. The elevation is 7,600 feet. In a region receiving an average of only 7 inches of precipitation per year, water is the lifeblood of the valley. The wet meadows, river oxbows, and riparian corridors support high wildlife diversity including songbirds, water birds, raptors, deer, beaver, and coyotes. Water resources on the refuge are managed to enhance wildlife habitat through an extensive system of ditches, water control structures, dikes, and levees. Land use surrounding the refuge includes cattle grazing, agriculture, and lands administered by the Bureau of Land Management (BLM).

The San Luis Valley has seven species of amphibians: striped chorus frog (*Pseudacris triseriata*), northern leopard frog, bullfrog (*Rana catesbeiana*), Great Plains toad (*Bufo cognatus*), Woodhouse's toad (*Bufo woodhousii*), plains spadefoot (*Spea bombifrons*), and tiger salamander (*Ambystoma tigrinum*).

Six new sites were monitored for metamorphs because either calling was heard, tadpoles or adults were previously observed, or because suitable breeding habitat was present. Sites are characterized as palustrine emergent wetlands (USFWS 1992a). The dominant cover species are spikerush (*Eleocharis* sp.), Baltic rush (*Juncus balticus*), sedges (*Carex* spp.), bulrush (*Scirpus* sp.), and cattail (*Typha* sp.). Submergent plants including pondweed and water milfoil are also present. The immediate surrounding land use is "water body/no riparian corridor."



## Monte Vista National Wildlife Refuge

Monte Vista NWR is located 6 miles south of the town of Monte Vista in the San Luis Valley. The elevation is 7,600 feet. The artificially created wetlands on the refuge's 14,804 acres are intensively managed to provide habitat for a wide variety of waterfowl and other water birds. Mallard (*Anas platyrhynchos*), pintail (*Anas acuta*), teal (*Anas* spp.), and Canada geese (*Branta canadensis*) are common, as are American avocet (*Recurvirostra americana*), killdeer (*Charadrius vociferous*), white-faced ibis (*Plegadis chihi*), egrets, and herons. The entire Rocky Mountain population of sandhill cranes (*Grus canadensis*) migrates biannually through the Monte Vista NWR. Irrigation canals and wells provide precious water to maintain important wetland habitat.

During the 2004 and 2005 seasons, reconnaissance was conducted to identify amphibian breeding habitat at Monte Vista. Viable breeding habitat during 2004, a drought year, was minimal. In 2005, five sites were identified as breeding locations and potential survey sites for chorus frog and Woodhouse's toad metamorphs. Chorus frog tadpoles were observed at three irrigation ditch sites and one emergent wetland. Woodhouse's toad juveniles were observed foraging in moist bottoms of two of the irrigation ditch sites. On return visits to the five sites, however, no amphibians were observed. Limited water and breeding habitat combined with substantial predatory pressure from waterfowl are presumed to be limiting factors to amphibian abundance at Monte Vista NWR.

## *Nebraska*

The Rainwater Basin (RWB) area in south-central Nebraska encompasses 17 counties, covers more than 4,200 square miles, and lies within the Central Mixed-Grass Prairie ecoregion within the U.S. Fish and Wildlife Service's (Service) Platte/Kansas Rivers Ecosystem. Prior to large-scale conversion to agricultural use, there were nearly 4,000 major wetlands totaling nearly 300,000 acres in the RWB area. To date, over 90% of these wetlands have been lost with less than 400 remaining. These wetlands have historically been, and currently are, important stopover and feeding locations within the Central Flyway for waterfowl and other migratory birds. Recognizing this fact, the Service created the RWB Wetland Management District (WMD) in 1963 to protect, restore, and manage wetlands and prairie grassland habitat along with native flora and fauna, including threatened and endangered species, waterfowl, and other migratory birds. Today, the RWB WMD manages more than 24,000 acres of land on 62 different properties (primarily consisting of Waterfowl Production Areas or WPAs).

Drought continued this year for much of Nebraska. The 12 sites monitored last were (2004) were monitored again this year. These sites consisted of the following 12 WPAs (with study site identification numbers in parentheses): Glenvil (RWB01), Harvard (RWB02), Mallard Haven – kiosk/cattle pond (RWB03), Mallard Haven – SW isolated wetland (RWB04), Theesen (RWB05), Smith (RWB06), Schuck – road ditch (RWB07), Schuck – pond (RWB08), Massie (RWB09), Moger (RWB10), Springer (RWB11), and Kenesaw (RWB12). Several of these sites dried up or were drying so new WPA sites were added. The majority of these WPAs have

current agriculture as a land use surrounding  $\geq 50\%$  of their boundaries. Corn is the primary agriculture crop. However, some fields are planted in a corn/soybean crop rotation. The primary contaminants of concern are pesticides (current use), fertilizers, and sediments.

Only four WPA locations had sufficient numbers of amphibians to be considered for monitoring. These four sites were Mallard Haven - SW (RWB04), County Line (RWB13), Waco (RWB17), and Schuck (RWB18). Mallard Haven was the only site that was monitored both this year and last year (2005). The four sites monitored are classified as emergent wetlands. Land use surrounding the sites, consist of current agriculture use, grassland/prairie habitat, and additional wetland habitat. The remaining eight locations were either dry, too difficult to sample due to site characteristics, and/or did not have enough tadpoles and newly metamorphosed frogs to warrant sampling.

### ***North Dakota***

Located on the Missouri Coteau (48° 37' N; 102°27' W), Lostwood NWR is a 109-km<sup>2</sup> area characterized by rolling hills of mixed-grass prairie and scattered aspen (*Populus tremuloides*) groves interspersed with a diversity of wetlands (Butcher 2003). Based on surface water samples taken in 2003, the U.S. Geological Survey documented pronounced levels of methyl mercury on the refuge (Johnson *et al.* 2003). Consequently, Lostwood NWR is now the locale for a 3-year study examining the trophic movement of mercury among various taxa (macro-invertebrates, amphibians, birds). Additionally, the study is examining the relationship between methyl mercury production in wetland landscapes containing prescribed burns versus unburned habitats. Since the early 1970s, prescribed burns have been conducted on Lostwood NWR to control woody vegetation and promote growth of native grasses (U.S. Fish and Wildlife Service 1998, Madden *et al.* 1999). However, burning is believed to exacerbate methyl mercury production in susceptible habitat types (Johnson *et al.* 2003). Monitoring of frogs for abnormalities at Lostwood NWR occurred in wetlands where prescribed burns occurred as well as in unburned habitats.

Three anuran species are known to occur on Lostwood NWR including the western (striped) chorus frog, northern leopard frog, and wood frog (Murphy and Danley *In Review*). Because chorus frogs are the most abundant species, data was collected on the metamorphs three sites in 2004. Unfortunately in 2005, very few tadpoles, few adults, and no metamorphs were found.

### ***Wyoming***

Mortenson NWR was established in May 1993 to protect the last known breeding population of the endangered Wyoming toad (*Bufo baxteri*). The refuge encompasses 719 hectares of the Laramie Plains and is located approximately 24 km southwest of Laramie, Wyoming in Albany County. The refuge is currently closed to all public access to minimize impacts to the Wyoming toads. The refuge contains four impoundments including Mortenson Lake, Garber Lake, Gibbs Pond, and Soda Lake. The majority of amphibians, including the Wyoming toad, tiger

salamander, and the western (striped) chorus frog, occur around Mortenson Lake because the other lakes are very alkaline. The northern leopard frog, formerly present in the Laramie Plains Basin, has been extirpated with no known reason. Mortenson Lake has an area of 32 ha and a well-developed wetland complex of bull rush (*Scirpus acutus*) and Baltic rush. Prescribed grazing is the primary land management tool. Mosquitoes are controlled on the refuge by *Bacillus thuringensis* var. *israelensis* (Bti) while the surrounding private property is sprayed with malathion.

Privately owned grasslands used for cattle grazing are located adjacent Mortenson Lake on the west and north. Land immediately south of the refuge is also grassland with a private home to the southwest. A dirt road and an adjacent lake with an emergent wetland fringe abut the refuge on the east side. The lake is on state land and designated for fishing. Although there are no regulations against motorized water craft, the lake is small and such watercraft use is not a frequent occurrence.

This was the second year of monitoring on the refuge. Various areas of the refuge were monitored for chorus frog tadpoles. However, we found very few tadpoles at two wetland sites (MRL01 and MRL02). MRL01 is classified as a wet meadow with shallow standing water (<30 cm). MRL01 was monitored in 2004 for metamorphs; but, we found no metamorphs in 2005. MRL02 is classified as a ditch with a muddy bottom and very shallow water (<5 cm deep or drying). The sides of the ditch were lined with emergent vegetation. Only one metamorph was found at MRL02



**Table 1.** Sampling locations on National Wildlife Refuges monitored for abnormal amphibians in 2005.

Refuge	State	Site Name	Sampling Season*	Potential Contaminants	Latitude	Longitude
Arapaho	CO	Fisherman Parking (ARP01)	2	Fertilizers, Nutrients	40.646111 °N	-106.479166 °W
Arapaho	CO	Germ Pond (ARP09)	2	Nutrients	40.739166 °N	-106.57422 °W
Arapaho	CO	Tour Loop South (ARP23)	1	Nutrients	40.6641 °N	-106.33353 °W
Arapaho	CO	Tour Loop North (ARP24)	1	Nutrients	40.66658 °N	-106.33263 °W
Arapaho	CO	Headgate on Illinois River North of HQ (ARP26)	1	Fertilizers, Nutrients	40.619388 °N	-106.282 °W
Alamosa	CO	ALM04	2	None suspected	37.374916 °N	-105.770222 °W
Alamosa	CO	ALM05	1	None suspected	37.36619 °N	-105.96373 °W
Alamosa	CO	ALM06	1	None suspected	37.36726 °N	-105.76457 °W
Alamosa	CO	ALM07	1	None suspected	37.38968 °N	-105.78041 °W
Rainwater Basin	NE	Mallard Haven WPA (RWB04)	2	Pesticides (current), Fertilizers, Sediment, Gravel Road Runoff (slight)	40.44301 °N	-97.73466 °W
Rainwater Basin	NE	County Line WPA (RWB13)	1	Pesticides (current), Fertilizers, Sediment, Other (feedlot)	40.69875 °N	-97.54501 °W
Rainwater Basin	NE	Waco WPA (RWB17)	1	Pesticides (current), Fertilizers, Sediment	40.90491 °N	-97.48265 °W
Rainwater Basin	NE	Schuck WPA (RWB18)	1	Pesticides (current), Fertilizers, Sediment	40.45683 °N	-97.99521 °W
Lostwood	ND		2	Heavy Metals, Nutrients		
Mortenson	WY	Mortenson Ditch (MRL02)	1	Pesticides, Nutrients	41.12557 °N	-105.49399 °W

\* Number of years that the refuge has been sampled.

## METHODS

Monitoring, sampling, and data collection were performed according to Standard Operating Procedures (SOPs) developed by Service's Chesapeake Bay Field Office and outlined in National Amphibian Malformation Monitoring on Refuges - 2001. Any variations from the standard SOPs by Service personnel conducting the amphibian monitoring are described under the appropriate state. In the years 2000 - 2002, any abnormal amphibians found were collected and sent to the National Wildlife Health Center (NWHC) in Madison, Wisconsin for examination by Dr. David Green to determine if the abnormality was the result of an injury or a developmental malformation.

Since 2003, live abnormal amphibians were sent to Dr. Dan Sutherland of the University of Wisconsin, La Crosse, for parasitology examination. Parasites, particularly the parasite *ribierioria*, have been suspected of causing some abnormalities in amphibians (Murphy et al. 1987). Dr. Sutherland recommended that it was best to send ten abnormal individuals from a site for parasitology work. However, if ten abnormal individuals were not found at a site possible, then the sample was completed (for a total of ten individuals) by substituting normal amphibians. This was to give Dr. Sutherland the best indication possible of the parasite fauna at a site. It was also recommended that when collecting amphibians, any snails that were caught should also be sent alive to Dr. Sutherland. The only confirmed hosts for *ribierioria* are planorbid "rams horn" snails. These snails may be a key link in elevated levels of *ribierioria* in wetland areas inhabited by frogs. Upon completion of the parasitology examinations, specimens were preserved in a 10% formalin solution for future radiographic studies.

### *Colorado*

#### Arapaho National Wildlife Refuge

The FWS Colorado Field Office (CFO) used the Standard Operating Procedures (SOP) for the monitoring effort with some additional information given below. The CFO was assisted by Pam Johnson, Refuge Biologist, and five seasonal biologists (Crystal Bechaver, Gene Hornyak, Tanya Manchester, Joy Nameth, and Lane Parkern). The refuge staff conducted reconnaissance on the refuge to identify amphibian breeding locations and to alert the CFO when metamorphs were ready to survey.

Surveys consisted of walking the site and looking for metamorphs. Nets were used to dip into standing water to look for tadpoles. Metamorphs were predominantly found along pond or wetland margins where soil was still very moist, or within very small pools of water. Metamorphs were captured by placing either a net or a cupped hand over the metamorph, then placing it in an open gallon-sized ziplock-type bag containing a small amount of water and vegetation. Metamorphs were transferred to either a 5-gallon bucket or a small cooler until they could be processed. Blue ice packs, 2 to 3 inches of pond water, and plenty of native vegetation were placed in the bucket or cooler, which was partially submerged in pond water to keep cool during collection. Capturing was conducted during the daylight hours. Captured metamorphs

were held in collection buckets until processing at either the hotel or onsite. Metamorphs were released to the same sites from which they were captured. Surveys took place during three separate field mobilizations, each of which spanned a period of two days.

#### Alamosa National Wildlife Refuge

The FWS Colorado Field Office (CFO) was assisted by Loree Harvey, a herpetologist with the BLM who lives in Alamosa. Prior to the CFO mobilizing a team to conduct surveys, Loree conducted reconnaissance on the refuge to identify amphibian breeding locations, and to alert the CFO when metamorphs were ready to survey.

Searches for metamorphs were conducted in the same manner as explained above for Arapaho NWR. Chorus frog and Woodhouse's toad metamorphs were found on the pond or wetland margins where soil was still very moist. Northern leopard frog metamorphs were found in shallow pond water and burrowed in sediment along the pond margins. Metamorphs were processed on the same day of capture and were released to the same sites from which they were captured. Surveys occurred on July 13 and 19, 2005.

#### Monte Vista National Wildlife Refuge

During the 2004 and 2005 summer seasons, reconnaissance was conducted to identify amphibian breeding habitat. In 2005, five sites were identified as breeding locations and potential survey sites for chorus frogs and Woodhouse's toads. Chorus frog tadpoles were observed at three irrigation ditch sites and one emergent wetland. Woodhouse's toad juveniles were observed foraging in moist bottoms of tow of the irrigation ditch sites. On return visits to the sites, no amphibians were observed.

#### *Nebraska*

Frogs were captured according to the Capture Protocol SOP with one modification. Once a frog was captured, it was placed in a large plastic ziploc bag along with a small amount of site water and/or some wet vegetation instead of being placed in a plastic tupperware container. The ziploc bag was used because it was easier to keep track of and the top portion could be placed in a pocket (with the bottom of the bag hanging out) where it was immediately accessible. Tupperware containers were not used because they were too big to stay tucked inside a set of hip or chest waders. Therefore, the tupperware container had to be placed on the ground and was not always immediately on hand. Additionally, using ziploc bags ensured that no frogs were injured by getting caught under a lid. Overall, the ziploc bags worked very well and did not have any observable adverse effects on the frogs. After several frogs had been collected, they were transferred to a bucket kept in the shade as described in the SOP. Data collection was conducted according to the SOP. Decontamination of field equipment occurred at the field office after all sites had been sampled according to the decontamination SOP. Used cleaning materials were disposed of at the field office. Abnormal frogs were shipped live to Dr. Dan Sutherland according to the Live Shipment SOP.



### *North Dakota*

During the summer of 2005, seventeen wetlands in Lostwood NWR were monitored for tadpoles during June and July. Several of these wetlands were monitored in 2004 for northern leopard frog tadpoles. The primary method of sampling the tadpoles was dip-netting. Gosner stages (Gosner 1960) of tadpoles captured in dip nets were characterized to estimate time that metamorphs would emerge from the wetlands and determine collection dates. However, no metamorphs were found during the monitoring effort.

### *Wyoming*

Monitoring for chorus frog eggs and tadpoles began in June and was repeated approximately weekly through mid-August following the SOPs. We used dip nets to monitor the Gosner stage of the tadpoles to estimate when metamorphosis would occur. Because of the difficulty in finding chorus frog metamorphs, we searched the sites by crawling on our hands and knees on several visits to ensure we would not miss any metamorphosing froglets. Unfortunately, during our monitoring efforts, we observed very few tadpoles and captured (by hand) only one metamorph for examination. Decontamination of field equipment occurred at the refuge after every visit according to the decontamination SOP. Used cleaning materials were disposed of at the Wyoming Field office in Cheyenne.

## **RESULTS**

### *Colorado*

#### Arapaho National Wildlife Refuge

Adult leopard frogs and chorus frogs were observed at site ARP01. No egg masses were observed. On August 5, 24 northern leopard frog metamorphs were captured and processed. One metamorph had a bent third digit on the right hind foot, but the metamorph was photographed and released. The other 23 metamorphs were normal and released after examination.

Eleven leopard frog metamorphs were observed at site ARP09. All metamorphs were normal and released after examination. At ARP23, 84 chorus frogs metamorphs were examined. All were normal with the exception of one that had a small laceration near the right eye. This metamorph was photographed and released. Fifty-six chorus frog metamorphs were examined at ARP24. Four metamorphs were considered abnormal. One metamorph had a missing hind right foot, one had a reddish bruise on the throat, one had a laceration and bruise on the left ventral site, and one was missing the entire left leg. The four abnormal metamorphs and six normal metamorphs were sent to Dr. Sutherland for parasitology. The remaining normal metamorphs were released. At ARP26, only four northern leopard frog metamorphs were found. All were normal and released after examination.

### Alamosa National Wildlife Refuge

Seventy-seven Woodhouse's toad metamorphs were captured at ALM04. All were normal and released after examination. Three chorus frog metamorphs were observed but not collected. At ALM05, 102 leopard frog, 56 chorus frog, and 61 Woodhouse's toad metamorphs were collected and processed. All were normal and released after examination. ALM06 dried up considerably during monitoring efforts. Only 12 northern leopard frog metamorphs were found. These metamorphs were all normal and released after examination. Fifty seven chorus frog metamorphs were captured and processed at this site. All were normal except one metamorph and the right hind foot missing. This metamorph and nine normal metamorphs were sent for parasitology. The remaining normal metamorphs were released after examination.

### Monte Vista National Wildlife Refuge

No metamorphs were observed.

### Nebraska

Sixty-six plains leopard frogs were captured at Mallard Haven WPA (RWB04). All frogs were in the process of final metamorphosis, with Gosner stages ranging from 42-46 (Gosner 1960). No abnormal frogs were found. A total of 53 plains leopard frogs were captured at County Line WPA (RWB13). Four abnormal frogs were found at the site. However, these abnormalities were most likely capture-related injuries. One frog (#4) was missing the tip of the second digit on its left hind foot. A second frog (#5) was missing the tip of the second digit on its left front foot. The base of the first and second digits was swollen and reddish. The third abnormal frog (#19) had a broken calf on the right hind limb. The skin was not broken, but the bone definitely was. There also were signs of internal bleeding in the immediate vicinity. The final abnormal frog (#37) was again missing the tip of the second digit on its left front limb. The digit was broken at the very end of the remaining tip. Additionally, the third digit of the same foot was bloody at the base and half-way up the digit. These four metamorphs were shipped live for parasitology with an additional six normal-looking frogs.

The Waco WPA was sampled two different times. On June 22, 2005, 101 unknown species of toad (*Bufo* sp.) were captured at Harvard WPA. These toads were most likely Woodhouse's toads (*Bufo woodhousii woodhousii*) given certain characteristics along with circumstantial evidence and knowledge of the biologist. However, since positive identification could not be made due to their small size and lack of development of certain distinguishing traits, they were categorized as an unknown toad species on the field data sheets and in this report. Three abnormal toads were found. The first toad (#33) was missing the first digit of its right hind limb. Additionally, the second digit (on the same limb) was broken and hanging. There were no signs of current injury (i.e., redness in the surrounding tissue). However, the remaining tip of the first digit looked a little swollen at the end like it was calloused or had a knobby end. The second toad (#58) was missing the first digit of its right front limb. Signs of internal injury were not mentioned on the data sheet. The last toad (#88) was missing the entire right front limb.



Additionally, there was a small circular tear in the outer layer of the skin where the limb should be; however, the body cavity was not punctured. There was no sign of internal bleeding. These three abnormal toads were shipped live, along with six normal toads, for parasitology.

On June 30, 2005, the Waco WPA was sampled a second time. Fifty-seven plains leopard frogs and 38 western chorus frogs (*Pseudocris triseriata*) were captured. No abnormal frogs were captured. Sixty-two plains leopard frogs were captured at Schuck WPA. There were no abnormal frogs found.

### ***North Dakota***

Seventeen Northern Leopard frog tadpoles (12 at Gosner stages 26-39, and 5 at stage 42) were found during the monitoring efforts. However, no metamorphs or adults were found during survey efforts.

### ***Wyoming***

Very few chorus frog tadpoles and only one metamorph was found during the monitoring effort. The one metamorph was examined, found to be normal, and released.

**Table 2.** Number of amphibians examined and number collected with abnormalities in 2005 from National Wildlife Refuges.

Refuge	Sample Date	Site Name	Common Name	Genus species	# Collected	# Abnormal	# Malformed
Arapaho	8/23/05	Fisherman Parking (ARP01)	Northern Leopard Frog	<i>Rana pipiens</i>	24	1	Abnormal specimen not collected
Arapaho	8/24/05	Germ Pond (ARP02)	Northern Leopard Frog	<i>Rana pipiens</i>	11	0	NA <sup>1</sup>
Arapaho	7/12-13/05	Tour Loop South (ARP23)	Western Chorus Frog	<i>Pseudocris triseriata</i>	84	1	Abnormal specimens not collected
Arapaho	7/12/05	Tour Loop North (ARP24)	Western Chorus Frog	<i>Pseudocris triseriata</i>	56	4	TBD <sup>2</sup>
Arapaho	8/24/05	Headgate (ARP26)	Northern Leopard Frog	<i>Rana pipiens</i>	4	0	NA
Alamosa	7/13/05	ALM04	Woodhouse's Toad	<i>Bufo woodhousii woodhousii</i>	77	0	NA
Alamosa	7/19/05	ALM05	Western Chorus Frog	<i>Pseudocris triseriata</i>	56	0	NA
Alamosa	7/19/05	ALM05	Northern Leopard Frog	<i>Rana pipiens</i>	102	0	NA
Alamosa	7/19/05	ALM05	Woodhouse's Toad	<i>Bufo woodhousii woodhousii</i>	61	0	NA
Alamosa	7/19/05	ALM06	Northern Leopard Frog	<i>Rana pipiens</i>	12	0	NA
Alamosa	7/13/05	ALM07	Western Chorus Frog	<i>Pseudocris triseriata</i>	57	1	TBD

Table 2. cont.

Rainwater Basin	7/06/05	Mallard Haven WPA (RWB04)	Plains Leopard Frog	<i>Rana blairi</i>	66	0	NA
Rainwater Basin	6/09/05	County Line WPA (RWB13)	Plains Leopard Frog	<i>Rana blairi</i>	53	4	TBD
Rainwater Basin	6/22/05	Waco WPA (WB17)	Unknown Toad Species	<i>Bufo</i> sp.	101	3	TBD
Rainwater Basin	6/30/05	Waco WPA (WB17)	Plains Leopard Frog	<i>Rana blairi</i>	57	0	NA
Rainwater Basin	6/30/05	Waco WPA (WB17)	Western Chorus Frog	<i>Pseudocris triseriata</i>	38	0	NA
Rainwater Basin	7/06/05	Schuck WPA (RWB18)	Plains Leopard Frog	<i>Rana blairi</i>	62	0	NA
Mortenson	7/26/05	Mortenson Ditch (MRL02)	Western Chorus Frog	<i>Pseudocris triseriata</i>	1	0	NA

<sup>1</sup>NA = Not Applicable.

<sup>2</sup>TBD = To Be Determined.

## DISCUSSION

### Colorado

Standard Operating Procedures may need to be modified for refuges in arid regions. Depending on the amount of moisture present in a given year, it may not be feasible in arid regions to collect the number of metamorphs per site that is currently recommended. Higher than normal snow pack and spring precipitation in 2005 increased surface water flows through the refuges as compared to 2004. The increased moisture resulted in an increase in favorable breeding habitat for amphibians in the 2005 season. This was the second year of surveying both Alamosa and Arapaho NWRs in Colorado. Overall, surveys were successful.

The availability of refuge staff to assist in the reconnaissance and daily checking of metamorph development was invaluable. Staff from the Colorado Field Office were better able to time site visits with the reconnaissance support from local and refuge staff, both FWS and contract.

### Nebraska

Upon review of the abnormal frogs and toads collected from County Line and Waco WPAs, the toad (#88) from Waco is the only individual frog or toad that is considered to be malformed. All other abnormal frogs and toads either exhibited obvious signs of injury or their abnormality consisted of just the tip of a digit missing. Although the final reports for the parasitology and radiology work have not been completed, Dr. Sutherland, the parasitologist, did call to say that the potentially malformed toad (#88) from Waco did not have an arm underneath its skin but did have a *Ribeiroia ondatrae* infection. *Ribeiroia ondatrae* is a type of trematode that is commonly found in leopard frogs, but is not as common in toads. This occurrence was the first documented occurrence of *Ribeiroia ondatrae* in toads from Nebraska.

The commonly accepted background rate of malformation for amphibians is 3%. All sites, except Waco WPA, had potential background malformation rates of 0%. Waco WPA had a potential background malformation rate of 1%. Last year, the Mallard Haven kiosk/cattle pond site, which is next to this year's sample site, had a potential malformation rate of 2%. However, it was noted that this potential rate could be misleading because only 50 plains leopard frogs were collected at the WPA last year. This year, the sample size was doubled and is, perhaps, more representative of the actual malformation rate. On the other hand, malformation rates can fluctuate between years and the difference between last year and this year may just be a fluctuation. Overall, it is important to recognize that Mallard Haven's malformation rates from both years were below the background rate.

Unfortunately, Theesen WPA was dry this year. This site was sampled last year and had a potential malformation rate of 3.9% for plains leopard frogs. However, only 51 frogs were captured. It would have been ideal to have two consecutive sampling years, but that could not happen. However, there are plans to sample the western half of the RWB WPAs starting in



2006. If that occurs, we will most likely try to monitor Theesen again and sample it a second time if there is enough water and frogs present. Otherwise, it appears that the eastern half of the RWB does not have a major concern with amphibian malformations.

### ***North Dakota***

We decided to sample for chorus frogs in 2005 due to abundance of chorus frogs and lack of northern leopard frogs found in 2004. Ironically, leopard frogs were the only species found in 2005. Chorus frogs were heard during breeding season but none were observed in mid to late summer. While tadpole leopard frogs were found, no metamorphs or adults were observed later in the summer.

Water levels likely were not a factor in the low frog numbers and incidents of observance in 2005. Water levels were stable throughout the summer. The ebb and flow of frog numbers we observed from year to year was also observed by Murphy and Danley (*In Review*). Amphibian monitoring during the summer of 2000 on Lake Alice NWR, in northeast ND, produced similar results. Frogs were readily abundant in 1999 and were inexplicably absent in 2000.

In light of the high incidence (14%) of potentially malformed chorus frogs found during surveys in 2004, and the complete lack of frog observances in 2005, it would be worthwhile to gather additional data through another season of malformation surveys at Lostwood NWR.

### ***Wyoming***

Chorus frogs at Mortenson Lake typically lay their eggs in early June with metamorphoses completed by late June or early July. Chorus frog eggs and tadpoles were observed at Mortenson on June 21, 2005. Two sites were checked regularly to monitor tadpole growth but tadpoles were few. With the exception of the one metamorph we found, no tadpoles that were any further along in development than having their hind limbs were observed. Surveys for metamorphs were successful in 2004 but the area surrounding MRL01 was burned in early spring 2005. It is unknown if this may have affected the chorus frog population but this was the only notable difference between 2004 and 2005.

## **CONCLUSIONS**

Assessment of amphibians for abnormalities on National Wildlife Refuges was successful in 2005 at refuges in Colorado and Nebraska but not in Wyoming or North Dakota. Overall, there were few abnormalities found in the amphibians. Determination of whether the abnormalities are deformities, malformations, or the result of parasites or injuries is pending.

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